RESEARCH

Sugarcane Leaf Photosynthesis and Growth Characters during Development of Water-Deficit Stress

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ABSTRACT

Yield and profitability of sugarcane (a complex hybrid of *Saccharum* spp.) grown on sand soils are much lower than on organic soils in Florida owing to biotic and abiotic stresses. A greenhouse study was conducted using a sand soil to identify effects of water deficit stress (WS) during sugarcane early growth on leaf photosynthetic components, plant growth, and dry matter accumulation. Treatments included two sugarcane genotypes (CP 01-2390 and CP 80-1743) and two water regimes (well watered [WW] and WS). All plants were well watered before initiating WS. Water was withheld from the WS pots when plants reached seven to eight leaves on the primary stem. During the WS treatment, plant growth and leaf photosynthetic components were measured. Final green leaf area (GLA) and shoot biomass were determined at the end of the experiment. Water stress depressed leaf relative chlorophyll level (SPAD), stomatal conductance (g_s), leaf net photosynthetic rate (Pn), transpiration rate (Tr), transpiration use efficiency (TUE) of photosynthesis, and GLA, resulting in reduced shoot biomass. CP 01-2390 was superior to CP 80-1743 in most measured physiological and growth traits under the WW and WS conditions, suggesting that selection of genotypes with tolerance to WS while improving irrigation management will improve sugarcane yields on sand soils. Physiological and growth traits, such as SPAD, g_s, Pn, Tr, TUE, GLA, tillering, and stalk length, may be useful for early detection of WS and for evaluation of sugarcane genotypes in the stress tolerance.

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Abbreviations: CP, Canal Point; CP program, CP sugarcane breeding and cultivar selection program; DRR, dark respiration rate; GLA, green leaf area; g_s, stomatal conductance; LCP, light compensation point; MQY, maximum quantum yield; PAR, photosynthetically active radiation; Pn, net photosynthetic rate; SPAD, Soil Plant Analysis Development; Tr, transpiration rate; TUE, transpiration use efficiency; TVD, top visible dewlap; WS, water stress; WW, well watered.

Sugarcane (a complex hybrid of *Saccharum* spp.) is an important crop with an economic impact of $3 billion in south Florida (Rice et al., 2009). Approximately 80% of this sugarcane was grown on organic (muck) soils and 20% on sand soils. A major goal of the Canal Point (CP) sugarcane breeding and cultivar selection program (CP program) in Florida is to develop high-yielding cultivars with disease resistance and tolerance to abiotic stresses for muck and sand soils (Glaz and Kang, 2008). Edmé et al. (2005) reported that for a 33-yr period, about 69% of the gain in sucrose yield of sugarcane in south Florida was from genetic improvements attributable to the CP program, but these yield gains were mainly associated with muck rather than sand soils. On the basis of these findings, scientists in Florida began a comprehensive review of the CP program to identify changes that can improve sugarcane yields for sand soils by both breeding and management practices without compromising progress on muck soils (Glaz et al., 2009).

Drought is one of the most important environmental stresses limiting sugarcane production worldwide (Venkataramana et al.,

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1986). Sugarcane grown on sand soils is often subjected to environmental stresses such as nutrient deficiencies (Ezenwa et al., 2005) and water deficit (Silva et al., 2007) caused by low soil organic matter and low soil water-holding capacity (Ezenwa et al., 2005) compared with muck soils in Florida. Thus, more emphasis on genotypic tolerance to the abiotic stresses that sugarcane faces on sand soils will contribute to improved genetic potential for yield. The CP program has been more successful for muck than sand soils (Edmé et al., 2005), perhaps mainly because of differences between the two soils in water availability and other environmental stresses. Development of genotypes tolerant to stresses, especially water stress during early growth, may improve sugarcane production on sand soils.

It is well known that water deficit stress alters a variety of physiological processes such as stomatal conductance, transpiration rate, leaf temperature, respiration, photochemical electron transport, and photosynthesis. These physiological processes are directly or indirectly associated with crop growth and yields (Silva et al., 2007). There is variation among crop species and among different growth stages of the same crop in response to water deficit stress. Four distinct growth stages (i.e., germination, tillering, grand growth, and maturity) have been characterized in sugarcane (Gascho and Shih, 1983). The tillering and grand growth stages, known collectively as the sugarcane formative growth phase, have been identified as the most critical water demand period (Ramesh, 2000), mainly because 70 to 80% of cane yield is produced in this phase (Singh and Rao, 1987). Therefore, plant water status, leaf photosynthetic components, and biomass accumulation during the formative phase can be useful in identifying sugarcane plant response to water stress.

Sugarcane physiological and morphological traits responsible for improved yield, sucrose content, and resource use are still poorly understood (Inman-Bamber et al., 2005). Improved understanding of traits responsible for improving water use efficiency and stress tolerance is needed to better develop and select elite genotypes (Edmeades et al., 2004; Inman-Bamber et al., 2005). On the basis of low yields and lack of genetic gain in sugarcane grown on sand soils (Edmé et al., 2005), it is probable that physiological processes are negatively affected by several abiotic stresses on sand soils, with water deficit stress being a major contributor to reductions in these physiological processes. Therefore, identification of physiological and growth responses of sugarcane to water deficit stress should help us better understand physiological mechanisms and improve cultivar selection and management practices for unfavorable environments such as sand soils in Florida.

Previous studies at multiple locations in Florida indicated that sugarcane CP 01-2390 ranked at the top among the 16 tested genotypes in both cane and sucrose yields, especially on sand soils (Glaz et al., 2007), but it is unknown what causes the yield differences, if some early growth and physiological traits are related to the final yield differences between CP 01-2390 and other genotypes, and how they respond a water deficit stress. To answer these questions, a 2-yr greenhouse study was conducted to investigate physiological and growth characteristics of sugarcane in its formative growth phase during development of water deficit stress on a sand soil. The specific objectives of this study were to: (i) identify water deficit effects on leaf photosynthesis components, plant growth, and dry matter accumulation and (ii) determine differences between two sugarcane genotypes in these traits during development of the water stress.

MATERIALS AND METHODS

Plant Culture and Treatments

The pot study was conducted in a greenhouse at the USDA-ARS Sugarcane Field Station, Canal Point, FL, in 2009 and repeated in 2010. Pots were 38 cm in both diameter and depth, with six small holes (1.5-cm diameter) at the base and filled with Margate sand soil (siliceous, hyperthermic Mollic Psammaquents). The sand soil was collected from a sugarcane production field near Clewiston, FL. Treatments included two water regimes [well watered (WW) and water stress (WS)] and two sugarcane genotypes (CP 80-1743 [Deren et al., 1991], a commercial cultivar for Florida muck soils, and CP 01-2390, an experimental clone with superior agronomic performance on sand soils in Florida). The filled pots were placed into individual containers that were used for desired water treatments. The photosynthetically active radiation (PAR) in the greenhouse was approximately 90% of ambient level without any supplemental lights. Greenhouse temperatures ranged from 30 to 35°C during the day and 20 to 25°C at night during the experiment. The night temperature in the greenhouse during the coldest period (January to mid-March) was relatively lower in 2009 than in 2010 because a heating system was installed in the greenhouse in 2010.

Fertilization with P (20 kg ha^{-1}), K (186 kg ha^{-1}), and micronutrients was performed at planting on the basis of soil test results and on recommendations for sugarcane nutrient management in Florida (Gilbert and Rice, 2009). Single-bud stalk sections of each genotype were planted in pots on 27 Jan. 2009 and 2 Feb. 2010. To ensure consistent conditions of soil water content and nutrients, the two genotypes were planted in each pot and labeled to indicate the genotypes. Thus, there were two plants per pot (one was CP 01-2390 and the other was CP 80-1743). A rate of 100 kg N ha^{-1} as a formula of ammonia nitrate was applied 22 d after planting. The rates of all fertilizers in this study were calculated on the basis of pot surface area. All pots were well watered from the top immediately after planting to reach the maximum soil water content and then by adding water in containers daily to keep an approximate depth of 2 cm of water before initiation of the water treatments. Thus, all pots were maintained near soil water capacity. Water stress treatment started 55 d after planting (24 Mar. 2009 and 29 Mar. 2010), when plants averaged 6.5 (2009) and 8.2 (2010) leaves on their primary stalks. Slightly bigger plants in 2010 than in

2009 at the initiation of the water treatments were mainly associated with the relatively high night temperature in 2010 as mentioned earlier. Water was withheld from the WS treatment pots and the water deficit stress gradually developed, while the WW pots continued to receive water daily.

Measurements

Soil organic matter content was determined before planting using the loss on ignition from 105 to 600°C. Soil total N and C contents were analyzed using a VarioMax CNS Macro Elemental Analyzer (Elementar Americas, Inc.). Soil bulk density and water-holding capacity were also determined. To estimate soil water-holding capacity and bulk density, three additional pots were filled with the same sand soil each year and transported to a laboratory. Excess water was added to the pots three times to completely saturate the soil. Extra water drained slowly from the small holes at the base of the pots. Soil cores were collected in each pot 24 h after water was added using a 0200 soil core sampler (Soilmoisture Equipment Corp.). The wet soil samples were thoroughly transferred to alumina soil cans from the brass cylinders and weighed. Then the wet soil samples were dried at 105°C for 24 h and weighed. Soil water-holding capacity and bulk density were calculated on the basis of the following equations:

$$\text{Soil water-holding capacity (\%)} = (\text{wet soil weight} - \text{dry soil weight})/\text{dry soil weight} \times 100$$

$$\text{Soil bulk density (g cm}^{-3}\text{)} = \text{dry soil weight (g)}/\text{core volume (cm}^3\text{)}.$$

The number of nodes (or leaves) and stalk length on the primary stalk and tillers were recorded in 3- or 4-d intervals from initiation of the WS treatment through the end of the experiment. Mean increment rates for main stalk elongation, nodes of the main stalk, and number of tillers were estimated on the basis of the following formula:

$$\textit{Mean increment rate} = \frac{(G_2 - G_1)}{(t_2 - t_1)}$$

where G_2 and G_1 are the growth parameters (i.e., the number of nodes, stalk elongation, or the number of tillers) measured at the ending (t_2) and beginning (t_1) dates of the WS treatments, respectively.

During the WS treatment, relative leaf chlorophyll level, leaf net photosynthetic rate (Pn), stomatal conductance (g_s), leaf transpiration rate (Tr), and photosynthesis transpiration use efficiency (TUE) were measured every 3 or 4 d between 0930 and 1400 h Eastern Daylight Saving Time from the top visible dewlap (TVD) leaves. Relative leaf chlorophyll level (soil plant analysis development [SPAD] reading) was estimated with a Minolta SPAD-502 chlorophyll meter (Minolta Co., LTD.). Leaf Pn, g_s, and Tr were measured using a LI-6400XT portable photosynthesis system (LI-COR Inc.). Photosynthesis TUE was estimated by dividing leaf Pn by Tr. When measuring leaf photosynthesis, PAR in the leaf chamber, provided by the 6400-40 LCF light source, was set to 1500 μmol m^{-2} s^{-1} with 10% of blue light; relative humidity was adjusted to near ambient level (60 to 70%); leaf chamber CO_2 concentration was set to 380 μL L^{-1}; and the flow rate to sample cell was set to 400 μmol air s^{-1}.

It is reported that photosynthesis light-response curves can be used to distinguish genotypes in response to drought stress in maize (*Zea mays* L.) (Liu et al., 2012). Therefore, in our study photosynthetic light-response curves of the TVD leaves were measured from three replicated plants in each treatment 10 to 12 and 7 to 9 d after the WS treatment in 2009 and 2010, respectively, using the "Auto Programs" of LI-6400XT portable photosynthesis system. When measuring leaf photosynthetic light-response curves, air temperature inside the leaf chamber was set to 32°C, relative humidity was set to 60 to 70% on the basis of the ambient humidity, and CO_2 concentration was set to 380 μL L^{-1}. The PAR was gradually decreased from 2000 to 0 μmol m^{-2} s^{-1} in nine steps (2000, 1500, 1000, 500, 200, 100, 50, 20, and 0 μmol m^{-2} s^{-1}). The photosynthetic light-response curves were fit using a least-square solution to a nonrectangular hyperbola according to Ögren and Even (1993). Values of leaf dark respiration rate (DRR), light compensation point (LCP), and the maximum quantum yield (MQY) of CO_2 assimilation were obtained by the initial linear regression of the last five levels of light intensities (PAR: 0, 20, 50, 100, and 200 μmol m^{-2} s^{-1}) and the respective gas exchange rates (Zhao et al., 2011). On the basis of linear regression, the absolute value of gas exchange rate, when PAR = 0, was defined as leaf DRR; the value of PAR, when gas exchange rate = 0, was defined as LCP; and the slope of the regression line was defined as MQY (Stirling et al., 1994; Zhao et al., 2011).

When leaves of the WS-treated plants clearly rolled up or wilted permanently, plants in all pots were cut near the soil surface and immediately separated into green leaves, brown leaves, and stalks. The numbers of large tillers (stem length ≥ 20 cm) and small tillers (stem length < 20 cm) were recorded. Green leaf area (GLA) was measured using a LI-3100 leaf area meter (LI-COR Inc.). The separated plant parts were dried in a forced-air oven at 60°C and weighed until their weights were stable.

Experimental Design and Data Analysis

The experiment was a two-factor factorial using a split-plot arrangement in a randomized complete block design with seven replications in 2009 and eight replications in 2010. Data were analyzed separately each year because of differences between years in plant size at the initiation of WS and the duration of the stress treatments. Replication was considered as a random effect, and genotype and water regime were considered as fixed effects. Date was considered as the repeated measurement for leaf SPAD and photosynthesis components. To test genotype, water regime, and their interactive effects on plant growth and physiological variables measured, significance of each fixed effect was analyzed using the MIXED procedure of SAS with covariance structure of compound symmetry (SAS Institute, 2003). If the hypothesis of equal means for measured traits between treatments were rejected and water regime × genotype interaction was significant by the *F* test, trait means were separated with the LSD at $P = 0.05$. The LSD values were calculated with the SE values generated by the Diff option in the MIXED procedure of SAS.

RESULTS AND DISCUSSION

Soil Properties

There were no differences between years in soil bulk density ($P = 0.326$), water-holding capacity ($P = 0.104$), pH ($P = 0.058$), organic matter ($P = 0.069$), C ($P = 0.103$) and N ($P =$

Table 1. Analysis of variance for leaf relative chlorophyll level (SPAD), leaf photosynthetic rate (Pn), stomatal conductance (g_s), transpirations rate (Tr), and transpiration use efficiency (TUE) in response to main effects of water regime, genotype, and measurement date and their interactions.

Year	Source	SPAD	Pn	g_s	Tr	TUE
				P > *F*		
2009	Water (W)	0.0616	<0.0001	<0.0001	<0.0001	<0.0001
	Genotype (G)	<0.0001	0.0364	0.0980	0.1295	0.1348
	W × G	0.3394	0.4452	0.3143	0.6303	0.6353
	Date (D)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	W × D	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	G × D	0.9840	0.1923	0.3130	0.4412	0.5598
	W × G × D	0.1067	0.8771	0.8110	0.9560	0.8300
2010	Water (W)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Genotype (G)	0.3946	0.6604	0.2195	0.3632	0.0755
	W × G	0.2484	0.8141	0.4297	0.5823	0.7102
	Date (D)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	W × D	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	G × D	0.3071	0.7709	0.1192	0.4299	0.0948
	W × G × D	0.3992	0.1345	0.4366	0.5183	0.6409

0.423) contents, and C/N ratio ($P = 0.168$). Their across-year mean values were 1.35 g cm^{-3} for soil bulk density, 20.1% for water-holding capacity, 6.9 for pH, 1.50% for organic matter, 7.75 g kg^{-1} for C content, 0.70 g kg^{-1} for N content, and 11.1 for C/N ratio. Soil used in 2009 had significantly ($P \leq 0.05$) lower acetic-acid-extractable P (25.7 mg kg^{-1}) and water-extractable P (2.5 mg kg^{-1}) contents, but higher K content (27.0 mg kg^{-1}) than soil used in 2010 (39.5, 4.0, and 13.9 mg kg^{-1}, respectively). Overall, water-holding capacity and N, C, and organic matter contents of the sand soil were much lower compared with those of organic soils in south Florida, and the stress symptoms of sugarcane plants appeared 7 to 10 d earlier on the sand soil than on the organic soil after a WS treatment (Zhao et al., 2010).

Leaf Relative Chlorophyll Level

Main effects of genotype in 2009, water regime in 2010, and measurement date and water regime × date interaction in both years on leaf relative chlorophyll level (SPAD reading) were highly significant (Table 1). During the WS treatment, leaf relative chlorophyll level of the WW plants declined slightly and did not differ between the two genotypes at most measurement dates (Fig. 1). In 2009 leaf relative chlorophyll levels did not differ significantly between the WW and WS plants for CP 01-2390 at all measurement dates ($P > 0.05$). In contrast, the WS treated plants of CP 80-1743 had significantly lower leaf relative chlorophyll levels than the WW plants 15 d after initiation of the WS treatment ($P \leq 0.05$). Under the 2009 WS conditions, CP 01-2390 had significantly higher levels of leaf relative chlorophyll than CP 80-1743 starting from 10 d after initiation of the treatment ($P \leq 0.01$). In 2010 relative chlorophyll levels of the WS treated plants sharply

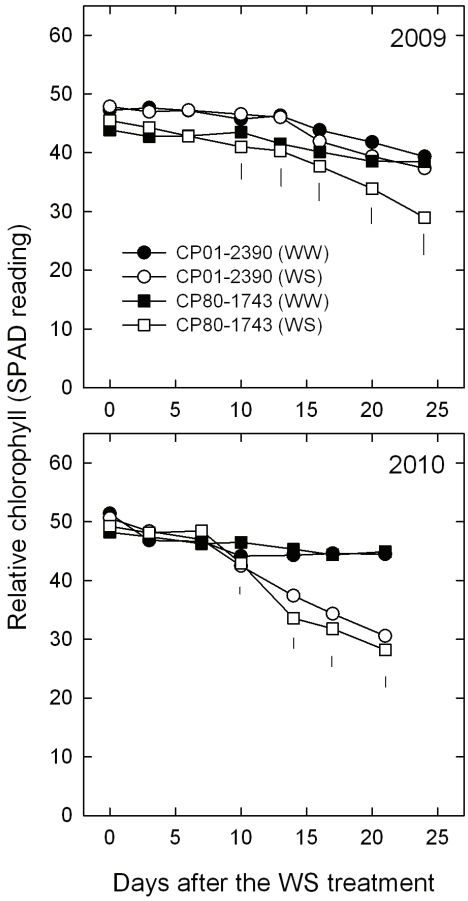

Figure 1. Relative chlorophyll levels (SPAD) of the top visible dewlap leaf measured with a SPAD meter as affected by genotype and water regime during the water deficit stress treatment. Data are means of six (2009) or seven (2010) replications. Vertical bars indicate $LSD_{0.05}$ values.

declined starting 7 d after the treatment. At 21 d of the WS treatment, leaf relative chlorophyll of the WS plants (29.4) decreased 34% compared with the WW plants (44.7), averaged across genotypes ($P < 0.001$). Under the WS condition between 14 and 21 d of the WS treatment, CP 01-2390 had a higher chlorophyll level than CP 80-1743 ($P < 0.05$), indicating that the latter had more severe stress.

Chlorophyll degradation is a consequence of drought stress that may result from photo-bleaching (Long et al., 1994). Our results suggested that starting from 10 (2000) to 15 (2009) d after initiation of the WS treatment, leaf relative chlorophyll level of the WS plants sharply declined, indicating plants were already exposed to the severe water stress at that time. Silva et al. (2007) found that drought caused a decline in sugarcane leaf chlorophyll level, but this reduction varied among genotypes. When sugarcane plants were exposed to the WS condition in the present study, CP 01-2390 had less reduction in leaf chlorophyll level than CP 80-1743 although the differences between the two genotypes in relative leaf chlorophyll level were much smaller than the differences between the WW and WS plants. On the basis of results of sugarcane leaf

chlorophyll level response to WS from the present study (Fig. 1) and from an early report (Silva et al., 2007), leaf SPAD readings can be one of the physiological traits to evaluate sugarcane genotypes for tolerance to WS.

Leaf Photosynthesis Characteristics

Water regime and measurement date significantly affected leaf Pn, g_s, Tr, and TUE (Table 1). The interactive effects of water regime × measurement date on these physiological traits were also highly significant ($P < 0.0001$). Genotype main effect was only significant on leaf Pn in 2009 ($P < 0.05$). There were no water regime × genotype, genotype × date, and water × genotype × date interactions in any of these photosynthesis variables (Table 1). Under the WW conditions, leaf Pn ranged from 28.0 to 35.8 μmol m^{-2} s^{-1} for CP 01-2390 and from 25.3 to 32.7 μmol m^{-2} s^{-1} for CP 80-1743 in 2009. In 2010 leaf Pn of the WW plants ranged from 28.3 to 37.7 μmol m^{-2} s^{-1} for CP 01-2390 and from 28.9 to 36.5 μmol m^{-2} s^{-1} for CP 80-1743 (Fig. 2A). Leaf Pn did not differ between the WW and WS plants in the first 7 to 10 d after initiating the WS treatment. Thereafter, leaf Pn of the WS-treated plants declined sharply and was significantly lower than that of the WW plants. Averaged across measurement dates, the WW and WS plants of CP 01-2390 had 7 and 11% higher leaf Pn, respectively, than the WW and WS plants of CP 80-1743 in 2009, but the statistical differences were not detected in 2010 (Fig. 2A). Clearly there were no differences in leaf Pn between the two genotypes under the severe WS (i.e., 7 d before the end of the study). Water deficit on sand soils in Florida under field conditions may not be as severe as the present pot study. Zhao and LaBorde et al. (2012) reported that CP 01-2390 had the highest leaf Pn among 14 tested sugarcane genotypes across the formative phase (April through July) under field conditions on sand soils, and it also had the highest sucrose yield at harvest (December). Therefore, high leaf Pn for CP 01-2390 during the formative growth phase on sand soils may partly explain the yield differences between sugarcane genotypes reported by Glaz et al. (2007).

The WS and measurement date significantly decreased g_s (Fig. 2B). Although linear relationships between leaf Pn and g_s were detected in both years ($r^2 = 0.80–0.96$, $P < 0.01$) for the WS plants during the experiment, g_s seemed to not be a major limitation of decreased Pn 15 to 24 d after initiation of the WS treatment because intercellular CO_2 concentration significantly increased during the second half of the stress period for both genotypes (data not shown). Decreased leaf Pn during moderate WS (i.e., the first half of the stress period) was associated with the decreased g_s, which is consistent with earlier reports (Du et al., 1998; Zhao et al., 2010). At the severe WS (20 to 24 d in 2009 and 10 to17 d in 2010 after initiation of the stress treatment), it has been suggested that reduced photosynthesis enzyme activities (Saliendra and Meinzer, 1991; Du et al., 1998) might mainly contribute to low leaf Pn because decrease in leaf Pn was much greater than the decrease in g_s. Leaf Tr (Fig. 2C) and TUE (Fig. 2D) for the WW plants fluctuated with measurement dates. There were no differences in either Tr or TUE between the two genotypes at most measurement dates. As expected no Tr and TUE differences were detected between the WW and WS treatments at the moderated WS. However, under the severe WS (last two measurements), leaf TUE was significantly decreased (Fig. 2D). When plants were exposed to WS, g_s and Tr declined, resulting in substantially reduced leaf Pn and TUE.

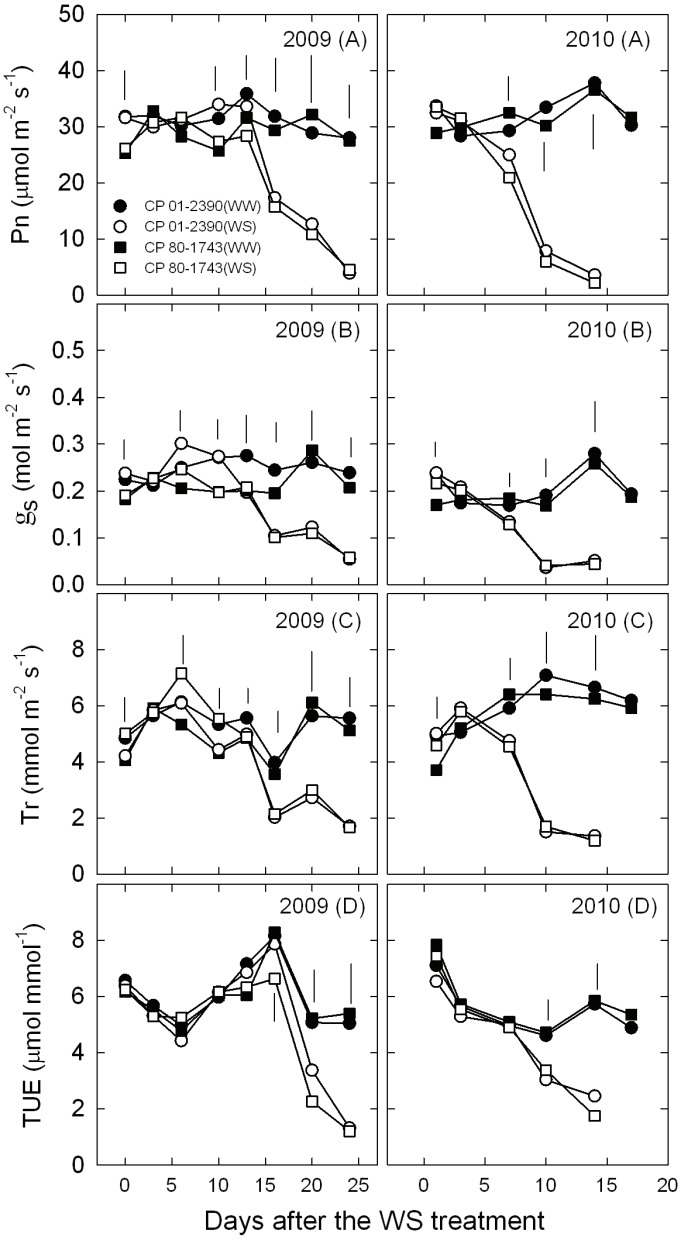

Figure 2. Responses of (A) leaf net photosynthetic rate (Pn), (B) stomatal conductance (g_s), (C) transpiration rate (Tr), and (D) transpiration use efficiency (TUE) to water regime during the water deficit stress treatment. Data are means of six (2009) or seven (2010) replications. Vertical bars indicate $LSD_{0.05}$ values.

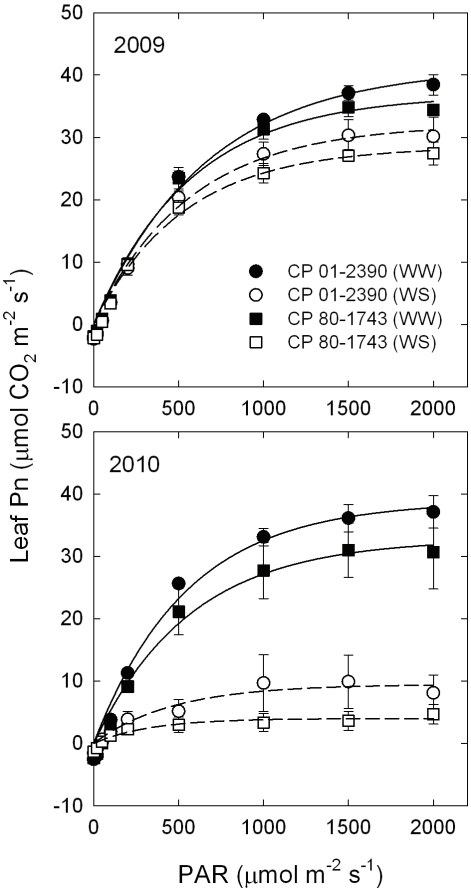

Figure 3. Responses of leaf net photosynthetic rate (Pn) to photosynthetically active radiation (PAR) as affected by sugarcane (a complex hybrid of *Saccharum* spp.) genotypes and water deficit stress. Data are means ±SE of three replicated plants for each treatment.

Leaf Photosynthesis Response to Photosynthetically Active Radiation

Leaf Pn responses to light intensity exhibited quadratic increases with increase in PAR for all treatments in 2009 and for the WW plants in 2010 (Fig. 3). As PAR increased from 0 to 1000 μmol m^{-2} s^{-1}, leaf Pn increased rapidly and thereafter, Pn increased slowly or leveled off as PAR increased. Leaf Pn did not differ statistically between the two genotypes within a water treatment at all other PAR levels in 2009, except for PAR levels of 1000 and 1500 μmol m^{-2} s^{-1} for the WS plants and 2000 μmol m^{-2} s^{-1} for the WW plants, at which CP 01-2390 had higher leaf Pn than CP 80-1743 ($P < 0.05$). In 2010 CP 01-2390 had significantly higher leaf Pn than CP 80-1743 at all PAR levels from 500 to 2000 μmol m^{-2} s^{-1} under both the WW and WS treatments (Fig. 3). Averaged across PAR (500 to 2000 μmol m^{-2} s^{-1}) in 2010, leaf Pn of CP 01-2390 and CP 80-1743 were 33.0 and 27.6 μmol m^{-2} s^{-1}, respectively, for the WW plants and only 8.2 and 3.7 μmol m^{-2} s^{-1}, respectively, for the WS plants.

The WS significantly depressed leaf Pn and the magnitude of Pn depression was associated with light intensity. The negative effects of WS on leaf Pn were greater under higher than lower PAR. When PAR was between 100 and 200 μmol m^{-2} s^{-1}, Pn of the WS plants did not differ from that of the WW plants in 2009, but the WS plants had 62% lower leaf Pn ($P < 0.001$) compared with the WW plants in 2010 at these PAR levels. At PAR of 2000 μmol m^{-2} s^{-1}, leaf Pn values of the WS plants in 2009 and 2010 were 21 and 81%, respectively, lower ($P < 0.01$– 0.0001) than those of the WW plants (Fig. 3). It is well known that WS affects both stomatal conductance and photosynthetic activity in the leaf (Taiz and Zeiger, 1998). Therefore, the effect of WS on photosynthesis includes both stomatic (restricted availability of CO_2) and nonstomatic (direct inhibition of photosynthesis) components. Munne-Bosch and Alegre (2000) reported that the efficiency of photosystem II photochemistry decreased to approximately 65% in *Melissa officinalis* plants exposed to the interaction of high light and drought.

Plant respiration is an important physiological variable and reflects the overall metabolism of plants (Flexas et al., 2005). Neither water regime nor genotype significantly affected leaf DRR, LCP, or MQY in 2009 (Table 2) because the plant size was relatively small with less consumption of water, and the WS was much less severe compared with the 2010 study. In 2010 WS significantly decreased leaf DRR and MQY but did not affect LCP. There was no genotype main effect or genotype × water interaction on any of these three physiological parameters. Averaged across genotypes, DRR of the WW and WS plants in 2010 were 2.90 and 1.06 μmol m^{-2} s^{-1}, respectively; their MQY were 0.065 and 0.022 μmol CO_2 μmol^{-1} photon, respectively (Table 2). The effects of WS on plant leaf respiration depend on the degree of the stress and crop species (Flexas et al., 2005). Our results indicated that sugarcane leaf DRR was not affected by moderate water stress in 2009 but significantly decreased under a more severe WS in 2010. Liu et al. (2012) reported that drought stress decreased leaf DRR and LCP in maize. Our results suggested that the WS significantly decreased sugarcane leaf DRR but did not affect leaf LCP.

Stalk Elongation and Node Addition, and Tiller Formation

At the beginning of the water regime treatment, CP 01-2390 had significantly longer stalk (Fig. 4A) and more tillers (Fig. 4C) than CP 80-1743, but the number of nodes did not differ between the two genotypes (Fig. 4B), and the same was true within a water treatment for all measurement dates. During the experiment, stalk length and the number of nodes increased linearly for the WW plants. Stalk length and number of nodes did not differ between the WW and WS plants on most measurement dates for both genotypes in 2009, probably because of the relatively small plant size. Starting from 10 d after initiating the WS treatment in 2010, the WS plants had significantly shorter stalks and fewer nodes than the WW plants. The WS plants

Table 2. Leaf dark respiration rate (DRR, μmol CO_2 m^{-2} s^{-1}), light compensation point (LCP, μmol photon m^{-2} s^{-1}), and maximum quantum yield (MQY, μmol CO_2 μmol $^{-1}$ photon) of CP 01-2390 and CP 80-1743 as affected by water deficit stress in 2009 and 2010.

Water regime†	Genotype	2009			2010		
		DRR	LCP	MQY	DRR	LCP	MQY
WW	CP 01-2390	2.35	39.11	0.059	3.09	43.24	0.071
	CP 80-1743	2.06	34.88	0.058	2.71	46.20	0.058
WS	CP 01-2390	2.46	43.24	0.058	1.17	48.25	0.027
	CP 80-1743	2.54	42.45	0.060	0.95	49.90	0.018
		P > *F*					
Water		0.300	0.088	0.983	0.002	0.618	<0.001
Genotype		0.683	0.417	0.922	0.469	0.790	0.115
Water × Genotype		0.503	0.571	0.758	0.853	0.940	0.776

†WW = well watered, WS = water stress.

had many fewer tillers than the WW plants from 20 (2009) or 15 (2010) d after initiation of WS (Fig. 4C).

Carr and Knox (2011) reviewed the water relations and irrigation requirements of sugarcane and summarized that leaf and stem extension was a more sensitive indicator of water stress than g_s or leaf Pn. When we estimated mean growth or increment rates for stalk elongation, node addition, and tiller formation during the experiment, water regime significantly affected rates of stalk elongation ($P < 0.01$) and node addition ($P < 0.05$) in both years and tiller formation ($P < 0.05$) in 2009 (Table 3). Genotype differences in the stalk elongation and tiller formation rates were also significant in 2009 but were not detected in 2010. That there was no significant genotype effect on tiller addition rate in 2010 is probably the result of the plants reaching near-maximum number of tillers during the WS treatment because of almost no changes in the number of tillers 10 to 22 d after initiation of WS for all treatments (Fig. 4C). There were no water regime × genotype interactions for any of these growth traits (Table 3).

Among these three growth parameters, stalk elongation rate was most sensitive to water supply. Compared with those of the WW plants, stalk elongation, node addition, and tiller formation rates of the WS plants were reduced by 40, 23, and 31%, respectively, in 2009 and 82, 62, and 37%, respectively, in 2010. Averaged across the two water treatments and years, CP 01-2390 had a 75% greater stalk elongation rate ($P < 0.01$), only 4% greater node addition rate (not significant, $P > 0.05$), and 76% greater tiller formation rate ($P < 0.05$) than CP 80-1743. These results indicated that CP 01-2390 was a well-adapted genotype on sand soils and grew much faster than CP 80-1743 on sand soils regardless of the water treatments (Table 3). It is noted that the height of plant canopy (from soil surface to top of plant canopy) was similar between the two genotypes within a water treatment, but CP 01-2390 had much longer stalks (from soil surface to TVD) and more tillers throughout the experiment. Therefore, growth traits of tillering and stalk length rather than canopy height may be useful for evaluation of sugarcane genotypes during early growth for sand soils.

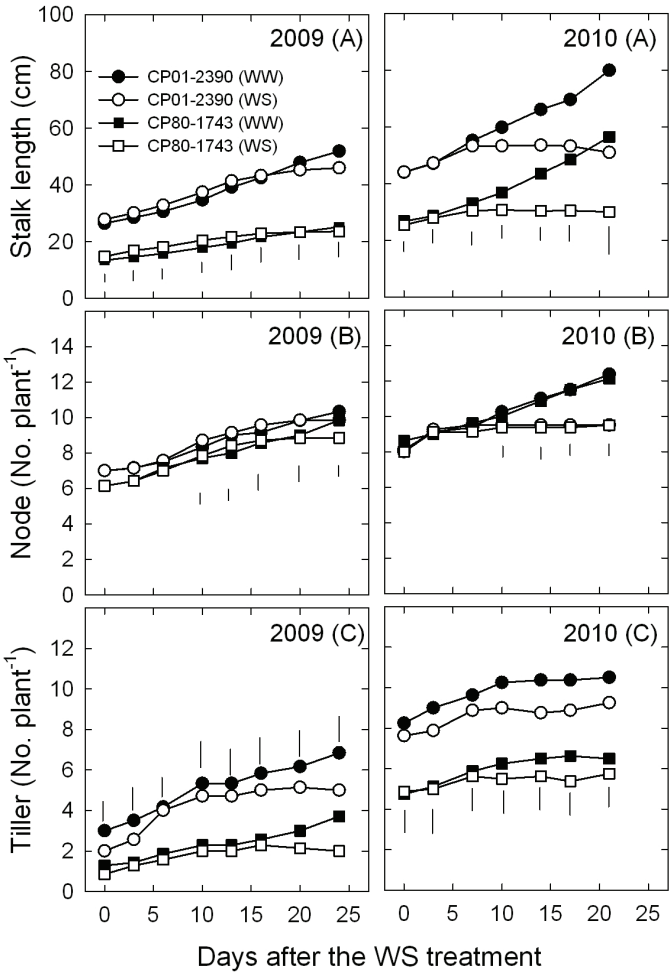

Figure 4. Changes in (A) primary stalk length, (B) the number of nodes on the primary stalk, and (C) total tillers for different treatments during the water deficit stress treatment in 2009 and 2010. Vertical bars indicate $LSD_{0.05}$ values.

Tillers and Green Leaf Area

Both water regime ($P < 0.05$) and genotype ($P < 0.01$) significantly affected the number of large tillers in 2009, but the number of small tillers was not affected by either the water regime or genotype ($P > 0.05$) at the end of the experiment (Table 4). There were no significant interactions of genotype × water regime on the number of tillers. Averaged across both genotypes in 2009, the WS plants reduced the large tillers by 47% compared with the WW plants. In 2010 WS decreased the number of large tillers by 37% but increased small tillers by 57%, resulting in no effect on total tillers. CP 01-2390 had many more total tillers than CP 80-1743 ($P < 0.01$–0.001) in 2010, and this difference was associated with more large tillers than small tillers (Table 4). Averaged across the water regimes and years, CP 01-2390 had 57% more large tillers, 27% more small tillers, and 44% more total tillers than CP 80-1743.

Table 3. Increment rates for main stalk elongation, node addition of the main stalk, and tiller formation of the well-watered (WW) and water-stressed (WS) sugarcane (a complex hybrid of *Saccharum* spp.) plants during the development of water-deficit stress in 2009 and 2010 as affected by genotype.

		2009			2010		
Water regime	Genotype	Stalk elongation	Node addition	Tiller formation	Stalk elongation	Node addition	Tiller formation
		cm d^{-1}	No. d^{-1}		cm d^{-1}	No. d^{-1}	
WW	CP 01-2390	1.06	0.14	0.16	1.71	0.20	0.11
	CP 80-1743	0.49	0.16	0.10	1.42	0.17	0.08
WS	CP 01-2390	0.66	0.12	0.13	0.33	0.07	0.08
	CP 80-1743	0.27	0.11	0.05	0.22	0.07	0.04
		$P > F$					
Water		0.004	0.029	0.047	<0.001	<0.001	0.106
Genotype		<0.001	0.707	0.004	0.065	0.119	0.173
Soil × Genotype		0.174	0.417	0.659	0.383	0.124	0.781

Table 4. Numbers of large tillers with stem length ≥20 cm, small tillers with stem length <20 cm, and total tillers and green leaf area at 28 (2009) or 22 (2010) d after initiation of water stress treatment as affected by water treatments and genotypes.

Water regime†	Genotype	2009				2010			
		Large tiller	Small tiller	Total tiller	Green leaf area	Large tiller	Small tiller	Total tiller	Green leaf area
		No. $plant^{-1}$			cm^2 $plant^{-1}$	No. $plant^{-1}$			cm^2 $plant^{-1}$
WW	CP 01-2390	3.17	3.17	6.33	3057	8.13	2.38	10.50	6351
	CP 80-1743	1.67	2.00	3.71	1172	4.25	2.25	6.50	3511
WS	CP 01-2390	2.29	2.71	5.00	1002	5.25	4.00	9.25	868
	CP 80-1743	0.29	1.57	1.86	269	2.50	3.25	5.75	795
		$P > F$							
Water		0.038	0.434	0.070	0.002	<0.001	<0.001	0.131	<0.001
Genotype		0.003	0.052	0.003	0.005	<0.001	0.153	<0.001	<0.001
Water × Genotype		0.611	0.985	0.752	0.179	0.271	0.302	0.698	<0.001

†WW = well watered, WS = water stress.

Water regime and genotype significantly affected GLA ($P < 0.01$- 0.001, Table 4). The interaction of water × genotype on GLA was not significant in 2009 but was significant ($P < 0.001$) in 2010. Although the WS significantly reduced GLA, CP 01-2390 had much greater GLA than CP 80-1743 under both the WW (161%) and WS (272%) conditions in 2009 (Table 4). In 2010 CP 01-2390 had 81% greater GLA than CP 80-1743 under the WW conditions, but no difference in GLA was detected between the two genotypes when plants were exposed to the severe WS. Sandhu et al. (2012) reported that leaf area index of field-grown sugarcane in mid-season was linearly related to cane yield. Therefore, greater leaf area and more tillers for CP 01-2390 than for CP 80-1743 during the formative growth under the WW and moderate WS conditions in the present study should be indicators of high yields on sand soils.

Shoot Biomass

Water regime ($P < 0.05$- 0.001) and genotype ($P < 0.001$) significantly influenced total shoot (green leaves + stalks + brown leaves) biomass, and their interaction was (2010) or was not (2009) significant (Table 5). Averaged across genotypes or across water regimes, the shoot biomasses of the WW and WS plants in 2009 were 34.8 and 21.5 g $plant^{-1}$, respectively, and the shoot biomass of CP 01-2390 and CP 80-1743 were 43.0 and 13.2 g $plant^{-1}$, respectively. In 2010, CP 01-2390 still had significantly greater total biomass than CP 80-1743 under the WW (89%) and WS (54%) conditions, although total shoot biomass had more reduction under the WS condition for CP 01-2390 (reduced 53%) than for CP 80-1743 (reduced 42%) compared with the WW plants.

Responses of green leaf biomass and stalk biomass to water regime were similar to the response of total shoot biomass to the WW and WS treatments, although the reverse pattern was found for brown leaf biomass response to WS. The biomass of green leaves dropped more than that of stalks under the WS condition. Compared with the WW plants, the WS plants had 70% less green leaf biomass and 45% less stalk biomass, but 186% greater brown leaf biomass, averaged across years and genotypes.

A 21- or 27-d WS treatment during sugarcane formative growth in our study significantly reduced stalk elongation and tiller formation on the sand soil. Similarly, total shoot biomass of the WS plants was reduced by 38% (2009) to 49% (2010) compared with the WW plants when averaged across the two genotypes (Fig. 3). On the basis of the growth

Table 5. Total shoot dry matter at harvest and shoot dry matter partitioning to green leaves, brown leaves, and stalks at 28 (2009) or 22 (2010) d after initiation of water stress treatment as affected by water treatments and genotypes in 2009 and 2010.

Water regime†	Genotype	2009				2010			
		Green leaf	Stalk	Brown leaf	Total	Green leaf	Stalk	Brown leaf	Total
		g plant^{-1}							
WW	CP 01-2390	23.94	25.66	1.87	51.46	50.75	59.64	8.07	118.46
	CP 80-1743	9.39	7.72	0.82	17.93	27.29	29.61	5.74	62.65
WS	CP 01-2390	10.75	16.77	7.02	34.54	7.84	28.19	20.01	56.04
	CP 80-1743	2.87	3.60	1.94	8.41	7.20	16.02	13.08	36.30
		P > *F*							
Water		0.001	0.024	0.022	0.020	<0.001	<0.001	<0.001	<0.001
Genotype		<0.001	<0.001	0.032	<0.001	<0.001	<0.001	<0.001	<0.001
Water × Genotype		0.212	0.377	0.120	0.482	<0.001	0.041	0.023	0.010

†WW = well watered, WS = water stress.

and biomass accumulation in the present study, CP 01-2390 performed much better than CP 80-1743 under both the WW and WS conditions on the sand soil in Florida. Reduced shoot biomass under the WS was associated with both low leaf Pn (Fig. 1) and decreased GLA (Fig. 4). The formative phase has been identified as the critical water demand period and the phase during which sugarcane is most sensitive to drought (Ramesh, 2000). Ramesh (2000) suggested that measurement of growth variables during the formative phase may help predict sugarcane total biomass at final harvest. It is evidence that the final yield differences between CP 01-2390 and other genotypes (Glaz et al., 2007) or CP 80-1743 (Zhao and LaBorde et al., 2012) were associated with most of the growth and physiological traits measured in the present study. In south Florida, the sugarcane formative phase usually occurs from February to July (Zhao et al., 2010). Precipitation in south Florida in the spring is much lower (a dry period of the season) compared with summer. Our results and weather characteristics in the region further suggest that water deficit during the formative phase may limit sugarcane growth and yields on sand soils. Thus, selecting cultivars more tolerant to drought during the formative phase while working to improve irrigation management, may improve sugarcane yield on sand soils in Florida.

Results of this pot study indicated that during formative growth, CP 01-2390 performed better than CP 80-1743 in most physiological and growth traits under the WW and WS conditions on a sand soil. In a recent study, Zhao and Comstock et al. (2012) reported that CP 01-2390 was a favorable parent for selecting offspring with high yield potential in several crosses that ranked in the top 20 in more than 300 crosses on the basis of growth vigor ratings and Brix values in the CP program. CP 01-2390 was not released as a cultivar for commercial use in Florida because of its susceptibility to smut [*Sporisorum scitamineum* (Syd.) M. Piepenbr., M. Stoll & Oberw.] disease even though it had superior cane and sugar yields (Glaz et al., 2007). In a more recent field study on sand soils at two locations in Florida, Zhao and LaBorde et al. (2012) found that even with smut infections in some plots, CP 01-2390 had the highest leaf Pn and cane and sucrose yields among 14 tested sugarcane genotypes. Therefore, CP 01-2390 was an elite genotype with high yield potential on sand soils in Florida, and crossing of CP 01-2390 with smut-resistant clones should be emphasized to generate new agronomically desirable combinations on sand soils in the breeding program.

CONCLUSIONS

Results of this study indicate that when sugarcane plants were exposed to increasing durations of WS, leaf relative chlorophyll level, g_s, leaf Pn, and Tr rapidly declined as the stress duration increased. The WS also significantly declined sugarcane leaf DRR and MQY but did not affect photosynthetic LCP. The negative effects of the water stress on leaf Pn under higher PAR were greater than under lower PAR. These reduced physiological parameters accompanied with small GLA for the WS plants resulted in a significant reduction in shoot biomass compared with the WW plants. CP 01-2390 was superior to CP 80-1743 in most measured physiological and growth traits during its formative growth on a sand soil under the WW and WS conditions. CP 01-2390 was also ranked the top among the 14 genotypes in leaf Pn and cane and sucrose yields under field conditions with sand soils at two locations in Florida (Zhao and LaBorde et al., 2012). The findings of the present study accompanied with the field investigations suggest that growth and physiological traits during tillering and grand growth can be used to predict sugarcane yield potential on sand soils in Florida. Selection of genotypes with tolerance to WS while improving irrigation management will help improve sugarcane yields on sand soils. Measurements of physiological and growth traits, such as g_s, Pn, GLA, tillering, and stalk length, may be useful for early detection of water stress and for evaluation of stress tolerant genotypes in the sugarcane breeding and cultivar development program to improve sugarcane yields and profitability on sand soils.

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product names is for informational purpose only and does not imply endorsement by the United States Department of Agriculture to the exclusion of any other product that may be suitable.

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